Research Progress about Nature of Early Pregnancy Factor

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Abstract Platelet-activating factor (PAF) exhibits a variety of biological activities and it be thought to involved in various pathophysiological process. In this paper, some studies were summarized about those roles of PAF in a variety productive processes of female of mammalian that include fertilization, implantation and parturition, and that was involved in the concentration, degradation and some assay methods of PAF. The relationship between PAF and early pregnancy factor(EPF was reviewed.
Key words: Early Pregnancy Factor, Chemical nature, Mammalian

Early pregnancy factor (EPF) is one of the earliest biochemical indicators of pregnancy (Morton et al., 1974). It has been detected within hours of fertilization being induced by the release of platelet-activating factor(PAF) from the fertilization ovum, and present for at least the first-two-thirds of pregnancy, continued detection being dependent upon the presence of a viable embryo or fetus (Orozco et al., 1986; Morton et al., 1987). This most interesting activity with potential applications in early pregnancy testing, for monitoring fetal well-being and in the study of fertility control, is detected in the rosette inhibition assay. When lymphocytes are exposed to heterologous red blood cells in the presence of complement, a small subpopulation of the lymphocytes binds red blood cells to form rosettes. The ability of these lymphocytes to form rosettes can be inhibited in a dose dependent manner by antilymphocyte serum (ALS), and so for a given ALS a rosette inhibition titre (RIT) may be defined, when lymphocytes are incubated in pregnancy sera prior to testing in this assay, an increased rosette inhibition titre is observed (Morton et al., 1976). The ability of pregnancy sera to cause this increase has generally been described (Morton et al., 1977, 1987) to the presence in these sera of a so-called 'early pregnancy factor'.

Research History of EPF

For 25 years the 'early pregnancy factor' phenomenon has defied molecular definition. First described in 1974 by Morton et al., this phenomenon was probably the first indication that there were significant communications between mother and embryo before implantation. The ability of early pregnancy sera to act onlymphocytes to cause increased rosette inhibition titres in the rosette inhibition assay has been studied for a considerable time(Smart et al., 1981; Whyte and Heap, 1983; Morton, 1984; Chard and Grudzinks, 1987; Morton et al., 1987). However, even now a complete molecular definition of this activity is not available, shortly after its discovery was ascribed (Morton et al., 1977) to the presence of an 'early pregnancy factor' in serum of pregnant animal, and this factor was assumed to be a pregnancy--specific protein(Rolfe, 1982; Morton, 1984, Morton et al., 1987; Mehta et al., 1989)

Notwithstanding some data indicating that a complex set of components may be insolvable (Clarke et al., 1980; Morton et al., 1980). Many attempts have been made to isolate EPF on the assumption that activity expression is due to presence of a simple pregnancy-specific protein. These include preparations from sera of pregnant ewes(Wilson et al., 1983), sheep placentae (Clarke et al., 1987), medium conditioned by oestrous mouse ovaries and oviducts stimulated with prolactin and medium conditioned by mouse embryos(Cavanagh, 1984), medium conditioned by the human choriocarcinoma serum (Mehta et al., 1989). They have produced a variety of results without any convincing identification or characterization(Yang, D.J. et al., 1995)

Some of the Molecules and Mechanisms Involved in EPF Activity

Studies on the nature of some of the molecules and mechanism involved in the rosette inhibition assay (Orozco et al., 1986; 1990; Clarke et al., 1990a, b) that allow the expression of increased rosette inhibition titres and also on some of the molecules and mechanisms by which pregnancy sera achieve this effect (Clarke et al., 1987; 1991; DiTrapani et al., 1991) have provided some new insights. In seeking to gain an appreciation of the real nature of the EPF phenomenon, it is just as pertinent to ask why non-pregnancy sera do not induce increased RITs as it is to ask why pregnancy sera do. This is so because some studies (Clarke et al., 1990) have revealed that many molecules (arachidonic acid and the leukotrienes, for example) which are normal serum

constituents(Beaubien et al., 1984; Hughes et al., 1989) can, in isolation, induce the expression of increased RITs. Moreover, it has been found that all sera can stimulate mouse spleencells to produce further active moieties (the S_2 factors) which have the capacity to induce increased RITs when applied to fresh, non-stimulated spleen cells. Thus, non-pregnancy sera possess not only endogenous active moieties, but also the capacity to stimulate the production of more during the assay procedure, yet they fail to induce increased RITs. This is so because these sera also induce a refractory state preventing the endogenous and induced active moieties from exerting their effects. This refractory state could be induced and maintained by any of a variety of potential stimuli and inhibitory or counteracting substances to be found in sera. Precedence for cells stimuli inducing a refractory state has been provided (Orozco et al., 1990) by the demonstration that PAF or calcium ionophore stimulation of mouse spleen cells renders them refractory to the action of the active S_2 factors, and specific examples of potential counteracting substance have been provided by the demonstration(Clarke et al., 1990b)that certain prostaglandins may counteract the action of leukotrienesin inducing increased RITs. Characterization of human (DiTrapani et al., 1991) and Ovine (Clarke et al., 1991) placental preparations, active in the rosette inhibition assay, revealed the presence of apolypeptide of relative molecular mass 12 kDa with associated active moieties of low molecular mass. Aminoterminal sequence analyses indicated that the proteins of Mr 12000 were vertebrate thioredoxins, an identification confirmed by cloning and sequencing of the cDNA Human recombinant thioredoxin has been expressed in Escherichia coli and purified. Pure thioredoxin alone does not induce increased rosette inhibition titres. However, when it is applied to spleen cells in combination with, or subsequent to such cell stimuli as platelet-activating factor(PAF) or normal serum, thioredoxin plays a permissive role allowing for the expression of increased inhibition titres, where none are achieved in its absence. It has been show to achieve this effect by preventing, or reversing, a refractory state induced in the spleen cell population by these stimuli, allowing lipoxygenase-dependent products, also generated in response to these stimuli, or possibly naturally present in the case of sera, to exert their effects in inducing increased rosette inhibition titres (Clarke et al., 1991).

Clarke et al.(1991) showed that all sera could stimulate the spleen cells used in the assay to produce potentially active moieties. However, as only sera of pregnant animals result in increased rosette inhibition titres, it has been suggested that sera from pregnant animals may be distinguished from that from non-pregnant animals by the presence of functional forms of thioredoxin or thioredoxin-like molecules, which act to reverse or prevent the refractory state induced by other serum components, and so allow for increased inhibition titres. Adsorption studies with anti-thioredoxin antibody(Clarke et al., 1991) support these conclusions and also indicate that there probably is an association in pregnancy sera (but not non-pregnancy sera) between the thioredoxin-like proteins and some active moieties of low molecular mass. Thus, it seems that the capacity to induce increased rosette inhibition titres is not due to the presence in sera of pregnant animals of some unique, pregnancy-specific protein but rather that all sera contain, or at least possess the capacity to generate mixtures of active and inhibitory moieties, that may induce increased inhibition titres depending on the relative proportions of these antagonistic moieties and presence or absence of specific functional forms of thioredoxin molecules that, if present, allow the expression of increased rosette inhibition titres.

Effect of Ammonium Sulfate on Early Pregnancy Factor Activity in Sera of Pregnant Animals

The susceptibility of early pregnancy factor activity in sera of pregnant animals to ammonium sulfate fractionation is well known(Clarke et al., 1980; Sueoka et al., 1989). Clarke et al. (1980) reported that when pregnancy sera were treated with 40% ammonium sulfate, and the resulting supernatant and pellet fraction were collected and extensively dialyzed and then tested in the rosette inhibition assay, neither pellet, nor supernatant fraction, could induce increased rosette inhibition titres. However these dialyzed fractions were recombined the combination induced increased inhibition titres, mimicking the action of the unfractionated sera. At the time, these data were taken to suggest that there were two parts to the system that allows pregnancy serum to induce increased inhibition titres. The supernatant fraction was said to contain the A-component(s)and the pellet fraction the B-component(s). Contemporaneous studies (Morton et al., 1980) on the tissues involved in production of early pregnancy factor activity suggested that the ovaries of pregnant animals produced components functionally equivalent to those in the pellet fraction from pregnancy sera, while the oviducts produced component(s) functionally equivalent to those in the supernatant fraction. As a result of these and other studies, it was suggested(Morton, 1984; Morton et al., 1987) that the proposed early pregnancy factor molecule was made up of two components or subunit. While discussion of this two component system Continues (but see

modified view of Cavanage et al., 1991), little definitive evidence to support this simple concept of early pregnancy factor has been forthcoming. Clarke et al.(1994) reinvestigated the effects of ammonium sulfate fractionation. Treatment of sera from pregnant mice with 40% ammonium sulfate was shown to liberate low molecular mass active moieties from their association with macromolecular serum components. After centrifugation, these active moieties partition into supernatant fraction, while the macromolecular components to which they are bound partition into pellet fraction. The macromolecular components of the supernatant and pellet fraction freed of any association with these low molecular mass moieties by extensive dialysis, can not alone induce increased rosette inhibition titres. In combination, however, component in the dialyzed pellet retentate fraction cooperate with components in the dialyzed supernatant retentate fraction to allow the expression of increased rosette inhibition titres when applied to fresh sheen cells in the rosette inhibition assay. In two-step incubation protocols, a prescribed order of addition must be followed if this cooperative effect is to be observed, namely supernatant retentate fraction applied first, followed by pellet retentate fraction in the second step, but not vice versa(Clark et al., 1994).

A New Model for the Basic System of Components Responsible for EPT Activity in Sera from Pregnant Animals

Orozco et al.(1994) present a model for the modes of action of the supernatant and pellet retentate fractions derived from sera from pregnant mice in cooperating to induce increased rosette inhibition titres, and to compare and contrast these action with those of the corresponding fractions derived from sera from male mice. The data clearly indicate that supernatant relentate fraction derived from sera from pregnant mice act in a manner analogous to that demonstrated for PAF(Orozco et al., 1990) and normal serum (Clarke et al., 1991), that is they stimulate the spleen cell population to produce active moieties(S_2 factors), but at the same time render the potential responding cell population(s) refractory to their action and thus when applied alone increased rosette inhibition titres are not observed. However, pellet retentate fractions derived from sera of pregnant mice act in a manner analogous to pure thioredoxin. Although ineffective when applied alone, if applied with or subsequent to appropriate cell stimuli such as PAF or the supernatant retentate fractions, they prevent or reverse the refractory state, allowing the expression of increased rosette inhibition titres. The capacities of supernatant retentate fraction derived from sera of pregnant mice to stimulate the production of active S_2 fractions and to cooperate with pure thioredoxin and the demonstrated capacity of the pellet retentate fractions derived from sera of pregnant mice to cooperate with a PAF stimulus are consistent with these conclusions.

The supernatant retentate fractions derived from sera from male mice would appear to be functionally equivalent to the corresponding fractions derived from sera from pregnant mice in that they also stimulated the production of active S_2 fraction and cooperated with both thioredoxin and pellet retentate fractions derived from sera from pregnant mice to induce increased rosette inhibition titres(Orozoco et al., 1994). The observation of these particular functional capacities in a fraction derived from sere from male mice is consistent with previous observations(Clarke et al., 1991) that unfractionated sera from male mice also express these capacities, that is the ability to cooperate with thioredoxin and to stimulate the production of an active S_2 fraction. The results reported by Orozco et al (1994) indicate that the component(s) responsible partition into the supernatant retentate fractions following ammonium sulfate fractionation of sera from male mice.

In contrast to the functional equivalence of the supernatant retentate fractions, the pellet retentate fractions derived from sera from male mice and from pregnant mice were not equivalent. Although the pellet retentate fractions derived from sera of pregnant mice function in a permissive manner in combination with PAF or the supernatant retentate fractions, those from sera from male mice do not(Orozco et al., 1994). This permissive capacity of the pellet retentate fractions derived from sera of pregnant mice was equivalent to that of pure thioredoxin, and this finding implies that functional thioredoxin may be present in these fractions. These conclusion are in agreement with previous suggestions(Clarke et al., 1991; Clarke, 1992) that a fundamental difference between sera from pregnant and non-pregnant mice may be the presence in the former of functional forms of thioredoxin or thioredoxin-like molecules.

The effect of ammonium sulfate on the capacity of sera from pregnant mice to induce increased rosette inhibition titres from the studies described by Clark et al. (1994) and Orozco et al.(1994) can be explained in principle in the following terms. Pregnancy sera contain stimulatory and permissive components and potentially active low molecular mass moieties, most or the most potent of which associate with the latter Sera from non -pregnant mice lack the permissive components and possibly the low molecular mass components. On ammonium sulfate fractionation of pregnancy sera, the low molecular mass

moieties are freed from their association with the permissive component and the low molecular mass moieties partition into the supernatant fraction with the stimulatory components. The permissive, thioredoxin-like components, partition into the pellet fraction. On dialysis the low molecular mass active moieties are removed from the supernatant fraction. The stimulatory fraction induces the production of potentially active moieties when applied to mouse spleen cells in the rosette inhibition assay but also induces a refractory state, and so alone can not induce increased rosette inhibition titres. On addition of the pellet retentate fraction which contains functional thioredoxin, orthioredoxin-like permissive molecules, this refractory state is reversed or prevented and the combination therefore allows for the expression of increased rosette inhibition titres. There is a considerable evidence to support the conclusion that functional thioredoxin or thioredoxin-like molecules are key components of the pellet retentate fractions of sera from pregnant but not from non-pregnant mice. With respect to the stimulatory components of the supernatant fraction and the low molecular mass active moieties, the studies reported by Orozco et al.(1994) establish their existence and relevance, and further studies are needed to establish their identity.

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